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Transmission patterns of African horse sickness and equine encephalosis viruses in South African donkeys

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SUMMARY

African horse sickness (AHS) and equine encephalosis (EE) viruses are endemic to southern Africa. AHS virus causes severe epidemics when introduced to naive equine populations, resulting in severe restrictions on the movement of equines between AHS-positive and negative countries. Recent zoning of South Africa has created an AHS-free zone to facilitate equine movement, but the transmission dynamics of these viruses are not fully understood. Here, we present further analyses of serosurveys of donkeys in South Africa conducted in 1983–5 and in 1993–5. Age-prevalence data are used to derive estimates of the force of infection, λ. For both viruses, λ was highest in the northeastern part of the country and declined towards the southwest. In most of the country, EE virus had a higher transmission rate than AHS. The force of infection increased for EE virus between 1985 and 1993, but decreased for AHS virus. Both viruses showed high levels of variation in transmission between districts within the same province, particularly in areas of intermediate transmission. These data emphasize the focal nature of these viruses, and indicate areas where further data will assist in understanding the geographical variation in transmission.

INTRODUCTION

African horse sickness (AHS) is a viral (Reoviridae: *Orbivirus*) disease of equines endemic to sub-Saharan Africa. Introductions to naive host populations elsewhere have caused severe epidemics. In the Iberian peninsula and Morocco it is estimated that 2000 horses died and >350000 were vaccinated from 1987–91 [1, 2]. The virus can infect most species of equines, although severe disease is usually found only in horses (up to 95% mortality). Based on its abundance and known vector competence, the biting midge, *Culicoides imicola* Kieffer (Diptera: Ceratopogonidae), is considered the most important vector [3–5]. This, however, does not exclude the possibility that other *Culicoides* species are involved. The North American species *C. sonorensis* Wirth and Jones is a competent laboratory vector [6, 7] while *C. bolitinos* Meiswinkel can be infected in the laboratory [8]. AHS virus has also been isolated from field collected *C. bolitinos* during an outbreak [9]. Several species of *Culicoides* can become infected with other orbiviruses, such as bluetongue and epizootic hemorrhagic disease [4, 10–13].

In South Africa, AHS virus is considered endemic in the northeastern parts of the country (primarily the Northern Province and Mpumalanga; Fig. 1) [14]. Outbreaks in southwestern South Africa appear to be the result of introductions of the virus to the local equine and *Culicoides* populations, followed by the loss of the virus when susceptible hosts are exhausted

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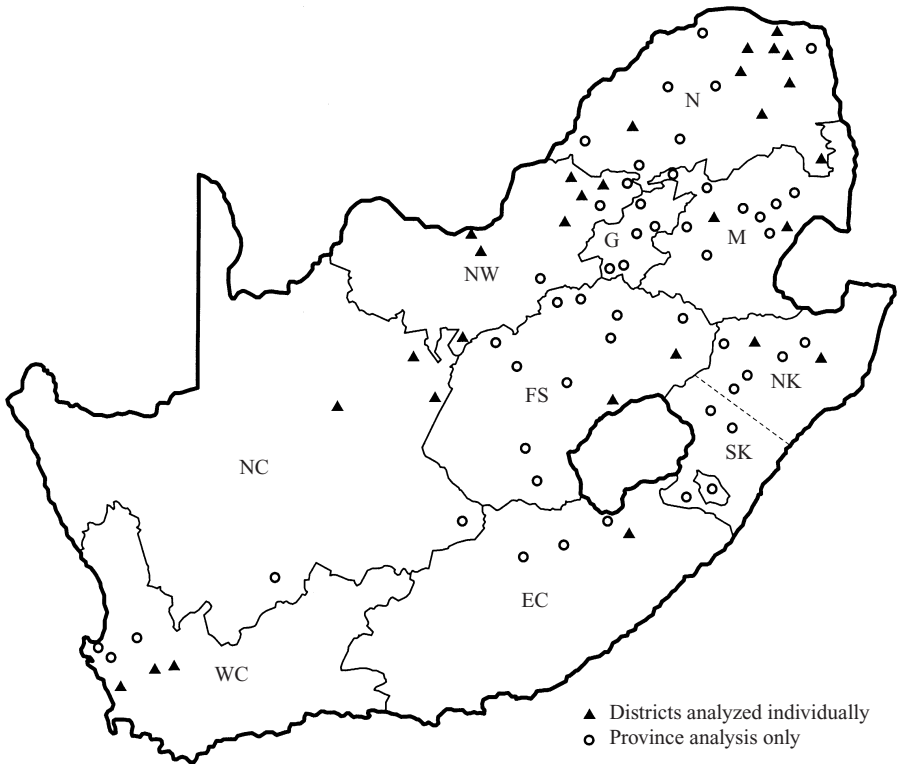

Fig. 1. Location of districts sampled in the 1983–5 serosurvey. Filled triangles; districts with sufficient samples to analyse separately; open circles, data used only in province and country-level analysis. NC, Northern Cape; WC, Western Cape; EC, Eastern Cape; NK, North Kwazulu/Natal; SK, South Kwazulu/Natal, FS, Free State; G, Gauteng; NW, Northwest; M, Mpumalanga; N, Northern. Note that Kwazulu/Natal is one province; North and South regions were considered separately due to expectations of different transmission rates.

or seasonal changes reduce midge populations. Zebra are the primary reservoir host [15], allowing circulation of the virus all year in areas with high zebra populations.

Until recently, all of South Africa, along with other countries in the Sub-Saharan area, was considered to be AHS – positive zones. This severely restricted the movement of equines in and out of these areas. This impacted the horse industry, preventing not only South African horses from being exported or competing abroad, but restricting the ability of the countries involved to host international equestrian events. The recent designation of an AHS-free zone in Cape Town (Western Cape) will aid in the exportation of horses from South Africa, but with significant costs in maintaining appropriate registration, vaccination and quarantine records. The location of the free zone was based on a historical absence of AHS, not the absence of vectors; difficulties with this approach were illustrated with the 1999 outbreak of AHS in the surveillance zone bordering the free zone [16]. Declaration of the small free zone still restricts the movement of animals within the country and limits the short-term visiting of horses for competition. The success of the free zone in allowing importation and exportation of equines, and any potential for expanding the free zone, will depend upon an in-depth understanding of how the transmission and epidemiology of AHS virus varies geographically.

Although very little is known about its epidemiology, equine encephalosis (EE) virus (Reoviridae: *Orbivirus*) is also endemic in southern Africa [17]. Similarly to AHS virus, EE virus appears to infect several species of equines, with disease found primarily in horses. There is a high seroprevalence in equines across the country [18], but a limited number of clinical cases have been reported; most cases are subclinical [17]. Although a variety of clinical signs have been ascribed to EE, few cases have been confirmed serologically or by virus isolation. *Culicoides imicola* has been shown to become infected and support replication of EE virus in the laboratory [19], and it is thought that *Culicoides* are the natural vectors [17].

Most horses in South Africa (except in the AHS free and surveillance zones) are routinely vaccinated

against AHS virus, while donkeys and other equines are rarely vaccinated. We would expect that vaccination of horses would decrease AHS transmission to donkeys, particularly in areas with few wild equines. No vaccine is currently available for EE virus; if the two viruses have similar transmission mechanisms, we would expect higher transmission of EE due to the lack of vaccination. A comparison of the transmission patterns of the two viruses in donkeys will provide insights into the transmission mechanism for EE virus and the effect of vaccination against AHS virus.

Much can be inferred about the transmission of a virus by examination of the prevalence of infection in different age classes of the vertebrate host [20]. The prevalence is often measured by the presence of antibodies, which measures exposure to the virus. In an endemic area, the typical pattern is a steady increase in the proportion of individuals infected with age. In areas with periodic epidemics, there should be sharp increases in the prevalence at ages corresponding to each epidemic. If transmission is rare and sporadic (infrequent introductions of the virus with insufficient vectors or hosts to maintain an epidemic), the prevalence should be zero in most age classes with low prevalence in some classes, but no apparent pattern.

The force of infection, λ, can be derived based on age-prevalence data in endemic areas [20, 21]. This provides a quantitative measure of the strength of transmission, which can be compared between areas or over time. Based on λ, the basic reproduction number, R_0, can also be calculated. R_0 is defined as the number of secondary cases resulting from one primary case in a completely susceptible population. From this definition, it is clear that if $R_0 \geqslant 1$, a virus can invade or persist in a population of hosts. If the type of transmission (e.g. endemic, epidemic, or sporadic) is due primarily to the size and seasonality of vector populations shared between AHS and EE viruses, we would expect values of λ for these viruses to be correlated across regions of the country. Vaccination would be expected to depress transmission of AHS, resulting in lower λ for AHS virus. If, however, the transmission mechanism for EE virus is not dependent upon the same or similar vectors as AHS virus transmission, we would expect no correlation between the force of infection for the two viruses.

Data are often grouped by political subdivisions, such as provinces, to estimate prevalence or force of infection in different areas. However, these political divisions may not reflect biological boundaries and may result in grouping data that are inherently inhomogeneous. Comparisons of the fit of age-prevalence data to the force of infection model at different spatial scales may reveal such groupings. Ideally, these types of data would also be analysed using spatial clustering methods to detect biological groupings and the appropriate spatial scale. In practice, however, the data may not be extensive enough to meet the requirements of these methods or the clusters may not be consistent between different clustering algorithms.

The climate varies considerably across South Africa, and we predict that this will result in variation in transmission of vector-borne viruses. The variation in climate will affect many aspects of viral epidemiology, particularly the ecology and population size of the vector(s). The west coast of South Africa is primarily hot, dry desert, changing to semi-arid plateau in the interior. The southwestern Cape has a Mediterranean climate (warm dry summer, cool wet winter), while the southeast coast is subtropical, with humid, wet summers. The temperate eastern plateau (most of Northern province and Mpumalanga) is cool, with wet summers and dry winters; becoming hotter and drier to the north.

The highest populations of *C. imicola* have been found at sites in the Northern province and northern Kwazulu/Natal [22], with low abundances in the interior of the Eastern Cape. However, trap catches varied widely over small spatial scales. Regression models developed from these data [23] indicated that soil moisture and annual minimum temperatures explained much of the variance in collections. These models predict low populations in the dry western interior, particularly along the Northern–Western Cape border, and high abundances along the coast and in the northeast. However, it should be noted that agricultural land use will also affect populations of *C. imicola*; the models did not address this factor.

Two data sets have been collected in the last 20 years on the prevalence of AHS and EE viruses in donkeys (*Equus asinus*) [18]. Basic analysis of the prevalence of the two viruses has been published [18]; we present here a further analysis of the data, the force of infection, λ, and the basic reproduction number, R_0, for different regions, and implications for the epidemiology of these viruses.

METHODS

Data

During two serosurveys, in 1983–5 and 1993–5, serum

samples were collected from donkeys and analysed for antibodies to AHS and EE viruses as described in Venter et al. [18]. Briefly, veterinarians were asked to collect blood from healthy donkeys which were still resident in their natal district. Attention was focused on younger animals; therefore, the sampled age distribution may not reflect the true age distribution. Only samples with reliable age data were used for this analysis. Sera were transported to the ARC-Onderstepoort Veterinary Institute and tested using ELISA [24]. Antibodies used were group-specific and not serotype-specific; therefore, no information was available as to the specific serotypes involved in positive reactions. For each sample, the magisterial district where the donkey resided was also recorded. Samples were grouped into monthly age classes for animals aged between 5 months and 1 year, and yearly age classes above 1 year. Samples from animals $\leqslant 5$ months old were not used, to avoid confounding with maternal antibodies.

The data were analysed at three geographical levels: country, province and district (Fig. 1). At the country and province level, all samples with reliable age data were used. The northern and southern parts of Kwazulu/Natal have different climates, and previous examination of the data indicated that transmission patterns might differ; therefore, the province was divided into two parts and these were analysed separately. Districts with $\geqslant 20$ samples and a prevalence $> 0\%$ and $< 100\%$ were analysed separately. No district had a sufficient sample size for independent analysis in the 1993–5 data set; therefore, only the earlier data were analysed at this level. Few districts were sampled in both surveys. All samples from a province were included in the province-level analysis regardless of whether the district was also analysed individually. Figure 1 shows the location of districts sampled in the 1983–5 survey. In addition, 11 samples were available from Windhoek, Namibia, in 1983–5. Routine vaccination of horses against AHS is less common in Namibia than in South Africa. Although the low sample size increases error, these data were analysed for a preliminary comparison of vaccinated and unvaccinated areas.

Force of infection

It was assumed that donkeys acquire infection with each virus with an age-independent constant force of infection, λ_i, e.g. the prevalence is a monotonically increasing function of age. The proportion infected at age a, $p_i(a)$, is, therefore,

$$p_i(a) = 1 - e^{-\lambda_i(a-M)}$$

where M is the age at which maternal antibodies become undetectable. Using maximum likelihood methods [25], λ_i can then be estimated as the value which maximizes the log likelihood,

$$\sum_a -x_{ia}\lambda_i(a-M) + y_{ia}\ln(1-e^{-\lambda_i(a-M)})$$

where x_{ia} is the number seronegative for virus i at age a and y_{ia} the number seropositive at age a. No data were available on M for donkeys; a value of 5 months was used based on data from zebra [15]. The variance of λ can be estimated as

$$\mathrm{var}(\lambda_i) = -\left[\sum_a -y_{ia}(a-M)^2 \times \left[\frac{e^{-\lambda_i(a-M)}}{1-e^{-\lambda_i(a-M)}} + \frac{e^{-2\lambda_i(a-M)}}{(1-e^{-\lambda_i(a-M)})^2}\right]\right]^{-1}.$$

The goodness of fit of λ_i for each serotype can be tested by likelihood ratios, comparing the maximum log likelihood (ML) with the likelihood from the fully saturated model (SM), calculated using observed values; $2^*(\mathrm{SM}-\mathrm{ML}) \sim \chi^2_{a,n-2}$ where n is the number of age classes. A common λ was fitted using the data for both viruses, and tested by likelihood ratios (as above) to the fit obtained with individual values of λ_is for each virus. A significant lack of fit in this test indicates that the λ_is are different. Similarly, a common λ was fit for each virus for all districts within a province, and compared to the individual values of λ_i for each district. If the prevalence of a virus in a district was 0%, a value of $\lambda = 0$ was used for comparison. Districts with prevalences of 100% were not included in the comparisons, as λ cannot be calculated. Provinces were compared to a common λ for the whole country in the same way. Programs for calculating maximum likelihood estimates of λ, goodness of fit tests, and comparisons between districts or provinces were written in Turbo Pascal.

95% confidence intervals were calculated for pairwise comparisons (estimated $\lambda \pm z_{0\cdot025}$*st. dev); non-overlapping confidence intervals are significantly different at the 5% level. This method assumes that the viruses are independently transmitted, that immunity is life-long (which is thought to be the case for AHS [14]), and that virus prevalence is in equilibrium. It also assumes that the host population is static (i.e. is not growing or decreasing and has a stable age structure).

R_0 for each virus is then calculated by

$$R_0 = \lambda_i(L-M)$$
$$\text{std } R_0 = (L-M)^* \sqrt{\text{var}(\lambda_i)}$$

where L is the average lifespan of the host. L was estimated as 5 years based on the age distribution of the samples and general information about the use of donkeys in South Africa. The linear relationship between R_0 and λ means that, for a given L and M, significant differences for λ also hold for R_0.

RESULTS

Province level analysis

The overall prevalence of each virus by province for each virus in the 1983–5 data set is shown in Figure 2. The prevalence of EE virus is generally higher than AHS virus, although similar patterns with respect to age are observed between provinces. This pattern is more obvious when λ is examined geographically (Table 1); transmission of both viruses is higher in the northeast and declines in the rest of the country. There were significant differences in λ both between provinces for each virus and between the two viruses within provinces (Table 1). There was significant lack of fit of the model for several estimates of λ (Table 1); the fit tended to be poorer when λ was higher.

λ was lower for AHS virus in the 1993–5 data set (Table 1, Fig. 3), significantly so in the Northwest province. For EE virus, however, λ was higher in the 1993–5 data set in all provinces considered, significantly so in Northwest and Free State provinces (Table 1, Fig. 3). The model did not show any significant lack of fit in the second data set (Table 1).

District level analysis

The 1983–5 data were sufficient for district level analysis in 28 districts in 7 provinces (Fig. 1). There was a high degree of variation between districts in some provinces, in particular those areas with intermediate levels of transmission (Fig. 4); λ was significantly different between districts in 5 provinces for AHS virus and 3 provinces for EE virus. Note that the confidence intervals are frequently large; this is due to the small sample sizes. There was significant lack of fit for the model in several districts (Fig. 4), indicating that the assumption of a monotonically increasing prevalence with age was not met. R_0 was greater than 1 in many districts (data not shown; AHS virus: 11 districts; EE virus: 22 districts), primarily in Northern Mpumalanga and the Northwest provinces.

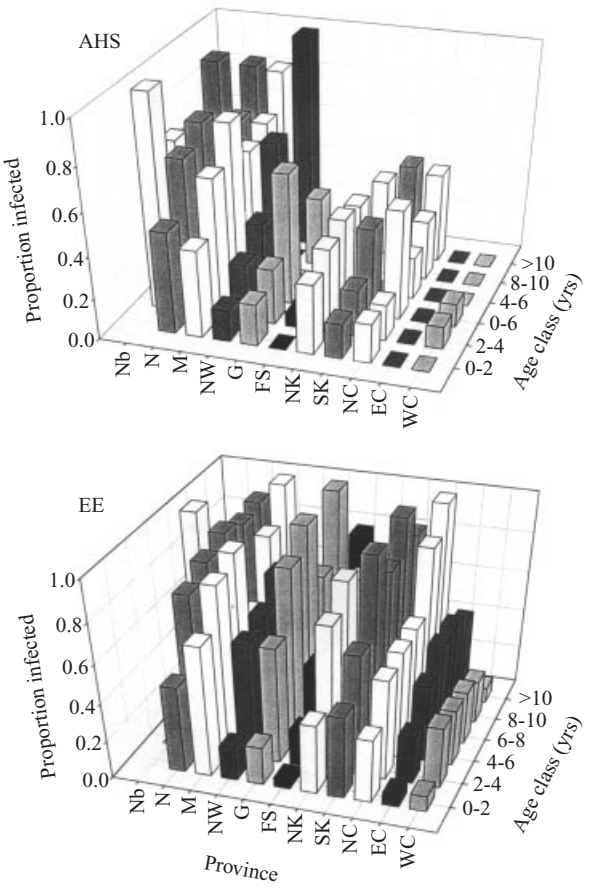

Fig. 2. Pattern of AHS (top) and EE (bottom) virus infection by age in each province for the 1983–5 data set. Abbreviations as in Fig. 1. Nb, Namibia. The Transvaal region encompasses the Northern and Mpumalanga provinces.

AHS vs. EE

In the 1983–5 data set, the transmission rates for EE virus were significantly higher than AHS virus in most locations tested, at both the district and province level. However, the transmission rates (and hence R_0) were highly correlated at both levels (provinces: $r = 0{\cdot}84$, districts: $r = 0{\cdot}9$, Spearman's rank test, $P < 0{\cdot}01$ for both; Fig. 5) This relationship did not hold in the limited data available from Namibia, here the transmission of AHS virus exceeded that of EE virus. Interestingly, the correlation was not significant in the 1993–5 data set (provinces: $r = 0{\cdot}6$, $P > 0{\cdot}05$); however, there were only four provinces with estimates of λ for both viruses.

DISCUSSION

The transmission of AHS and EE viruses clearly vary across South Africa. As was expected, higher trans-

Table 1. *Estimated force of infection for AHS and EE viruses in provinces of South Africa; 1983–5 and 1993–5*

	AHS				EE			
	1983–5		1993–5		1983–5		1993–5	
Province	λ	*n*	λ	*n*	λ	*n*	λ	*n*
Northern*	0·47 ± 0·04[e]	296	0·29 ± 0·06	39	0·56 ± 0·05[e]	262	0·67 ± 0·16	38
Mpumalanga†	0·31 ± 0·04[e]	133	nd[a]		0·55 ± 0·07[e]	129	nd	
Northwest†*‡§	0·14 ± 0·02[e]	241	0·06 ± 0·01	141	0·29 ± 0·03[e]	221	1·04 ± 0·22	99
Gauteng†	0·10 ± 0·03	42	nd	0	0·30 ± 0·06[e]	43	nd	
Free State§	0·01 ± 0·01	98	0·01 ± 0·00	79	0·10 ± 0·02	73	0·60 ± 0·15	34
North Kwazulu/Natal†	0·08 ± 0·02	85	nd	0	0·22 ± 0·03[e]	87	nd	
South Kwazulu/Natal†	0·06 ± 0·02	54	nd	0	0·34 ± 0·07	46	nd	
Northern Cape†*	0·08 ± 0·01[e]	99	0·02 ± 0·01	25	0·23 ± 0·04	77	0·29 ± 0·11	11
Eastern Cape†	0·0[b]	43	0·0	13	0·07 ± 0·02	41	nd	5
Western Cape†	0·01 ± 0·00	111	0·0	20	0·05 ± 0·01[e]	94	high[c]	18
South Africa[d]	0·13 ± 0·01[e]	1202	0·06 ± 0·01	317	0·25 ± 0·01[e]	1073	0·70 ± 0·08	205

λ: estimated value ± st. dev; n: number of sera used in estimating λ.

[a] nd; analysis not done; either no samples from province or too few to warrant analysis.

[b] $\lambda = 0$, none of the samples were positive; 0 used in comparisons.

[c] All samples were positive; λ could not be calculated but was high.

[d] Samples from all provinces combined.

[e] significant lack of fit of model.

λ significantly different between viruses: †1983–5 *1993–5.

λ significantly different between data sets: ‡AHS §EE.

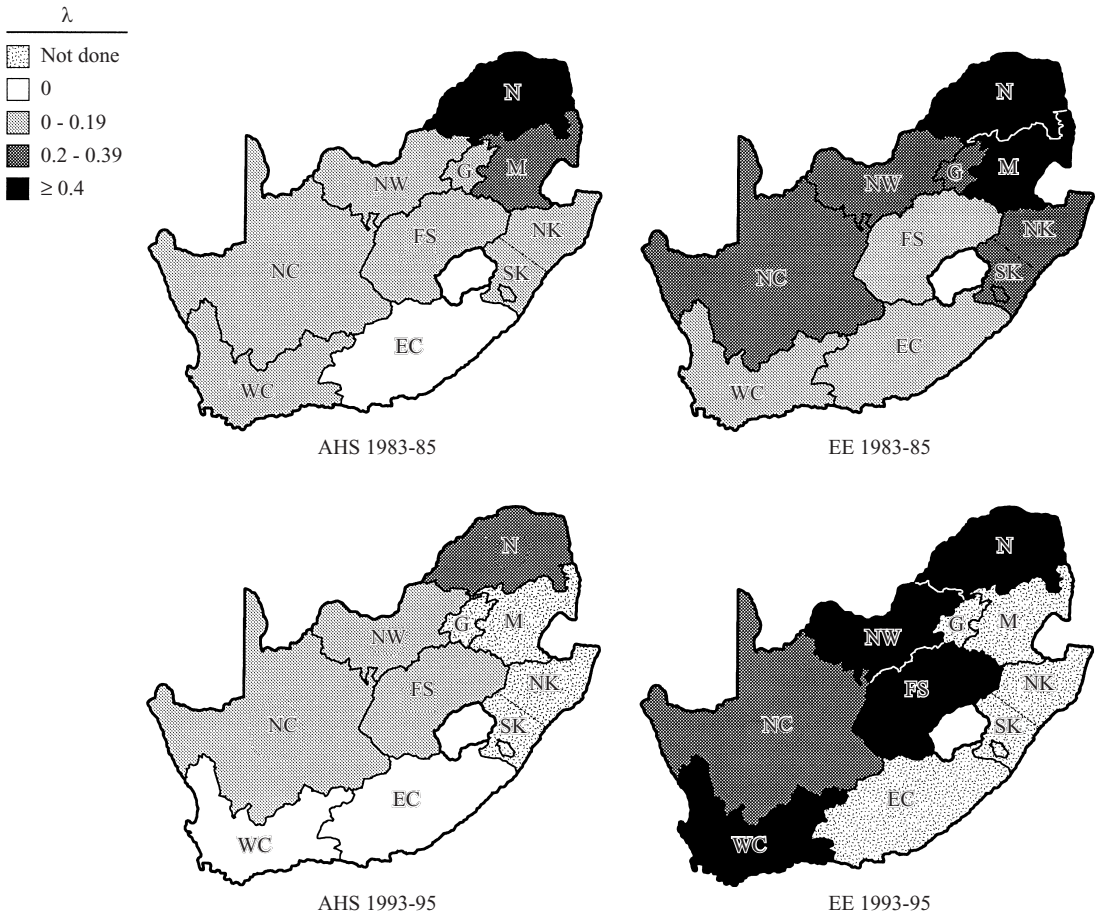

Fig. 3. Map of λ values by province for both viruses and both data sets. Exact values and errors in Table 1. Provinces with 100% prevalence were assigned to the highest λ category.

mission occurred in the warmer northeast. Zebra in this area experience very high transmission of AHS virus [15, 21], and serve as a reservoir [15]. The transmission patterns would suggest that zebra also serve as a reservoir for EE virus, but further work is required to confirm this. The data analysed here did not include identification of the serotypes of AHS virus, as all nine serotypes circulate in the zebra population [15, 21] it is likely that multiple serotypes would be present in the donkey population as well. Multiple serotypes also occur in EE virus [17], but there are few data available on the patterns of serotype prevalence. Frequently, outbreaks of AHS outside the endemic areas are of only one or two serotypes at a time [5]; serotype data from donkeys would be informative in tracking outbreaks of individual serotypes through space and time.

Estimates of λ indicate that the Northern province, Mpumalanga, and parts of the Northwest province have higher transmission of both viruses than the rest of the country. The values of R_0 indicate that both AHS virus and EE virus can be sustained in these areas ($R_0 > 1$). This is, however, sensitive to the estimates of M and L used, longer average lifespans or shorter durations of maternal antibodies would raise the estimates of R_0. Areas in the South African interior and Kwazulu/Natal show patterns consistent with epidemic transmission, while the Cape provinces appear to have only rare, sporadic transmission. Data were lacking in many areas in the middle and western parts of the country, however; it is difficult to identity the boundaries of these zones.

The data from Windhoek, just inside Namibia to the north of Northwest province, indicates some of the impact of vaccination against AHS virus and the potential for increased AHS virus transmission if vaccination pressure was released; transmission of AHS virus was higher than in either of the adjoining South African provinces.

These transmission patterns, which are similar between AHS virus and EE virus, indicate that aspects of transmission are shared between the two viruses.

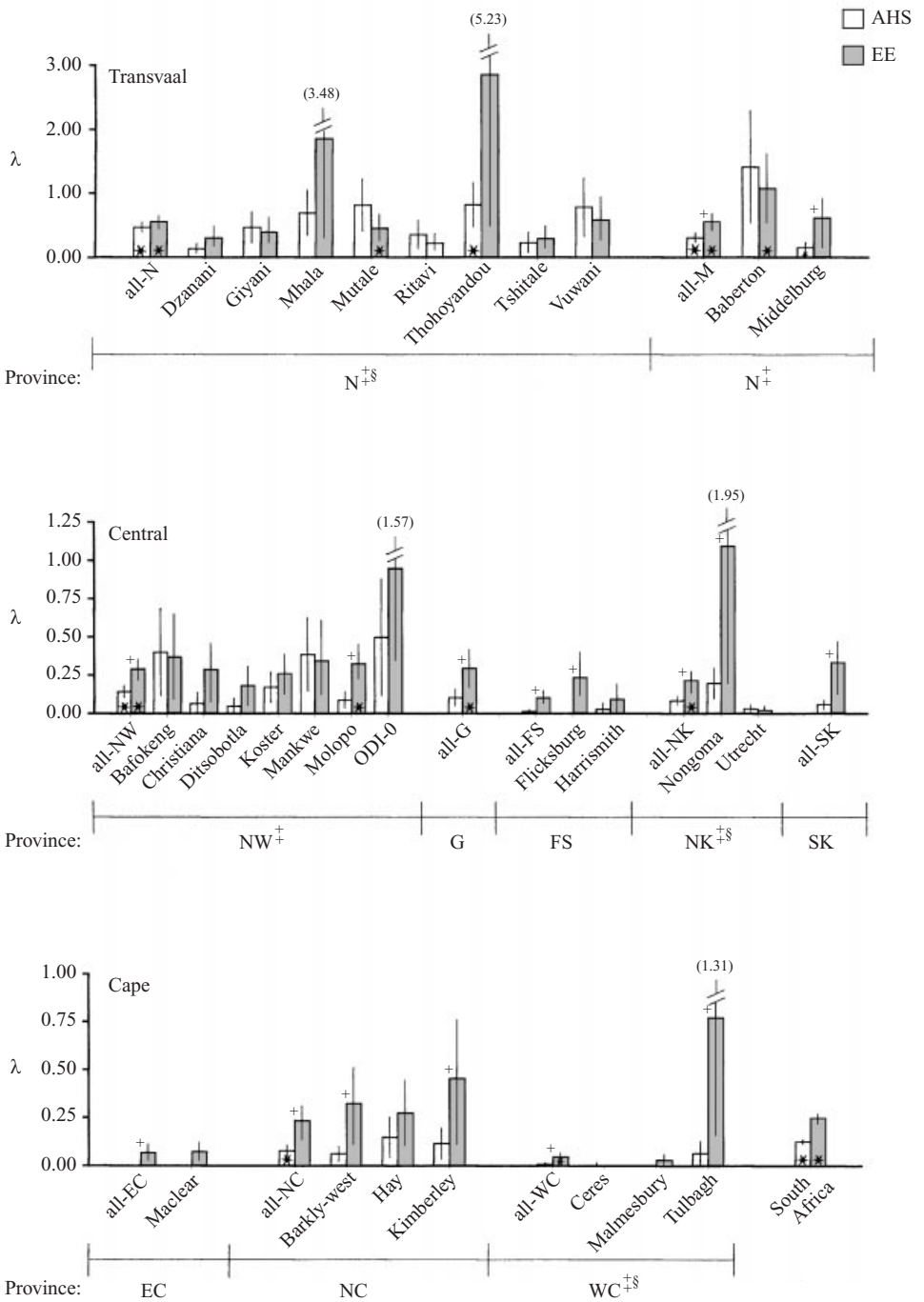

Fig. 4. Variation in λ by districts. Bars show $\lambda \pm 95\%$ confidence interval; estimates with non-overlapping confidence intervals between viruses in one location or between districts within a province for one virus are significantly different. Province abbreviations as in Fig. 1. ‡, districts significantly different within province for AHS virus, § for EE virus. +, AHS and EE viruses significantly different; * , significant lack of fit of model.

The most likely explanation, as has been previously suspected [17], is that the two viruses are transmitted by the same vectors. The population size of the vector has been shown to be an important factor in many modeling studies of vector-borne disease, including AHS [20, 26]. The geographical abundance pattern of *C. imicola* predicted based on satellite imagery [23] is consistent with the geographical patterns observed in these data, although the model used for prediction depends upon the presence of livestock and may not be accurate in some parts of the country. Comparisons of the geographical distribution of various *Culicoides* species, historical records of AHS cases, and transmission rate data would be valuable. Epidemics in the

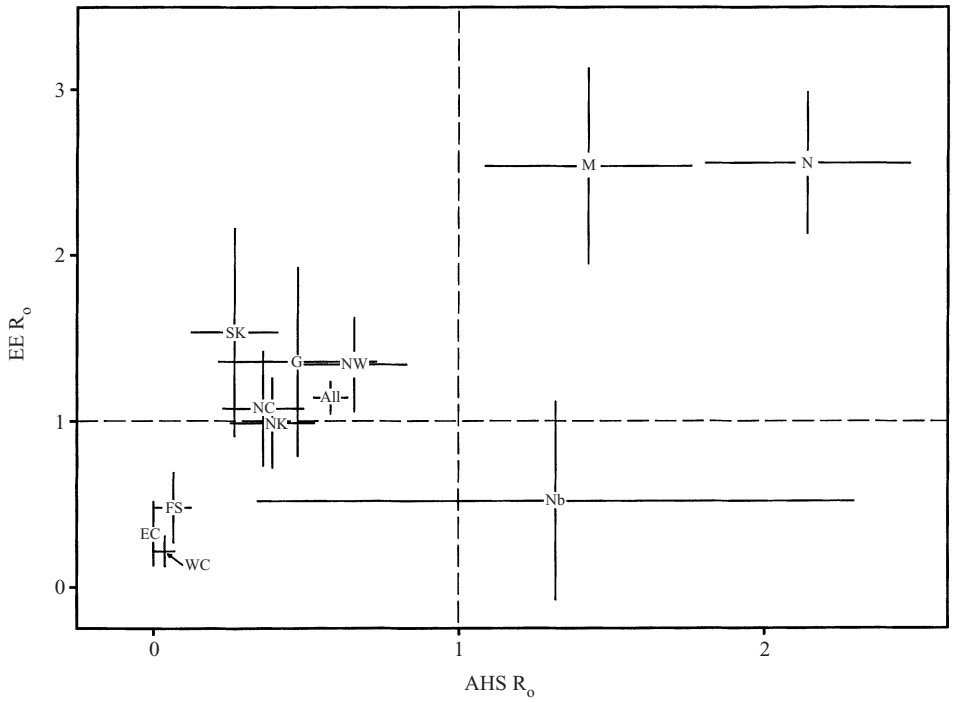

Fig. 5. Correlation between R_0 for AHS and EE viruses, by province, in the 1983–5 serosurvey. Province abbreviations as in Fig. 1. Nb, Namibia; all, all samples from South Africa.

central and western parts of South Africa clearly depend on many factors. Recently, outbreaks of AHS virus have been linked to El Niño/Southern Oscillation events [27], most likely due to the effect of weather patterns on *Culicoides* abundance and zebra migration patterns.

Clearly, the simple model of a constant force of infection was not appropriate for all of the data. At the province level, the lack of fit of the model used is, in part, due to the heterogeneity of transmission and pooling data over large regions. The model was generally better supported at the province level when the districts were not as different or the data came from fewer different districts (Table 1, Fig. 4). Pooling of data over heterogeneous groups, whether the groups are defined by geography, age, or other factors, may lead to problems in fitting models [21, 28]. This could also be a factor in the lack of fit of the model in some districts; analysis of transmission in some areas may require a finer spatial scale than was possible with these data. Preliminary attempts to use cluster analysis on these data failed; there were insufficient data to identify reliable clusters. Sample sizes sufficient for analysis at the district level (or finer scale) for more locations, particularly in areas with high heterogeneity in prevalence, would be required for detailed spatial analysis. This type of data and analysis would be valuable in understanding the epidemiology of AHS and EE viruses in South Africa.

Another consideration for the lack of fit of the model is sample size, particularly in the older age classes. The sampling of donkeys was deliberately biased towards younger animals [18]; this could affect the parameter estimates. Small sample sizes will obviously skew prevalence data, and will affect the goodness of fit of models. There may, however, be epidemiological concerns not adequately considered by this model. Using age-prevalence data for estimation of λ depends upon the assumption of a monotonically increasing prevalence with age; this may not be appropriate in areas with sporadic or epidemic transmission. In these areas, we would expect to see no infection in young age classes (animals born after the last outbreak), with a constant prevalence in older age classes (those exposed to past epidemics). In addition, the duration of the immune response in the absence of viral challenge is not known in donkeys. If older animals loose immunity, we would expect an age-prevalence curve which initially rises, then falls with age. Visual inspection of the data from provinces and districts where the model exhibited significant lack of fit, however, did not show the patterns expected from either of these hypotheses. It is most likely, therefore, that the lack of fit of the model to these data

is primarily a function of pooling heterogeneous data and small sample size in older age classes.

If the transmission mechanisms for the two viruses are the same, the higher transmission rates of EE virus can be used to indicate the potential transmission of AHS virus in the absence of vaccination. Routine vaccination is not allowed in the newly-designated free and surveillance zones in the Western Cape province. As the equine population becomes more susceptible, introductions of AHS virus could become more damaging. The 1999 outbreak of AHS in Stellenbosch (Western Cape, in the surveillance zone) illustrated the costs of outbreaks in a susceptible population. Widespread vaccination of animals in the surveillance zone, necessary in outbreaks, increases the difficulty in monitoring possible transmission and decreases the security of the free zone. Expansion of the AHS-free zone (and associated control zones) will require further data on the transmission of AHS virus in the Cape provinces. The apparent focal nature of virus transmission, evidenced by the variation between districts, indicates that monitoring and further data should be collected at multiple locations within each province. Monitoring of EE virus in the donkey population will also provide information about *Culicoides* – transmitted arboviruses in the equine population.

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